LABORATORY Spotlight

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The National High Magnetic Field Laboratory

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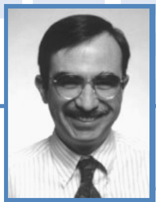

Attention Users

Tim Cross
NMR Program Director

A unique resource is being installed at the NHMFL in Tallahassee for our NMR Spectroscopy and Imaging users. One of the original goals laid down by the NSF was to achieve magnetic fields in excess of 25 T for NMR spectroscopy. In its first phase towards that goal, the NHMFL is now placing into its final cryostat a magnet designed for 900 MHz (21.1 T) having a room temperature bore of 105 mm. In a recent *NHMFL Reports* (Spring 2002), a thorough report through the early stages of bucket testing was presented. Now, following several months of these tests, the Magnet Science and Technology Group is ready to weld the magnet into its final dewar where the LHe will be at 1.8 K. Full field, high homogeneity, and high stability for NMR experiments ranging from solution and solid state NMR to imaging, diffusion, and *in vivo* spectroscopy are anticipated. Here, I would like to sketch, in general terms, a draft of the scientific vision and its justification for the use of this unique magnet.

The NMR Spectroscopy and Imaging Program at the NHMFL is a national and international user program with access to instruments free of charge. Its mission is to develop technology, methodology, and strategies for very high field applications and to develop the frontiers of the application science through its users and technological developments. Nearly 100 research groups participated in this mission in the past year at these facilities in Tallahassee and in Gainesville at UF. Strong radio frequency efforts are in place at both sites to facilitate the research of both local and external users to accomplish unique spectroscopic and imaging results.

High fields have many advantages:

- Well known enhancements in sensitivity are proportional to $B_O^{7/4}$, resulting in a decrease in signal averaging time proportional to $B_O^{7/2}$.
- Also well known are improvements in resolution at high fields. At constant line width (in Hz), resolution improves linearly with field strength (B_O) for each chemical shift dimension of an NMR experiment.
- Relative magnitudes of field-dependent and field-independent spin interactions change with magnetic field strengths resulting in a reduction of the second-order broadening of quadrupolar nuclei that is linearly dependent on field.
- Magnetic susceptibility effects increase as B_O^2 having important implications for functional MRI, including improvements in spatial resolution and sensitivity.
- Magnetic susceptibility is also responsible for the partial magnetic alignment (as B_O^2) of macromolecules in solution leading to a lowered dependence on liquid crystalline additives for obtaining residual dipolar interactions. Magnetic susceptibility also contributes to the uniform alignment of samples for biological solid state NMR.
- The field dependence of relaxation parameters can lead to improved linewidths (in Hz) and potentially to the use of multiple contact cross polarization at very high fields, thus increasing sensitivity by two, three, or four-fold.
- Increased field strengths provide a greater range of resonance frequencies sensitive to the molecular frequencies of macromolecules, thereby enhancing NMR's ability to provide detailed characterizations of molecular dynamics.
- The relative influence of field-dependent and field-independent relaxation mechanisms can lead to greatly enhanced resolution through the TROSY experiment at high fields for ^{15}N resonances. This experiment at high field will make available NMR as a structural and dynamic characterization tool for many high molecular weight systems and complexes.

- At high frequencies there is a reduction in probe ringing that avoids long delays for detection of signals with short T_2 relaxation.

While these are many of the predicted benefits of high fields, it has been the unpredicted benefits of high fields that are today transforming the NMR community's strategy for macromolecular structure determination and magnetic resonance imaging.

- Spectroscopists over the years have warned that multiple relaxation mechanisms would complicate high-field NMR leading to severe disadvantages. Today, we recognize how to make use of constructive interference between different relaxation mechanisms at high magnetic fields in order to turn this disadvantage into a means for investigating much larger molecules through an NMR experiment called TROSY.
- Until recently, most spectroscopists ignored the slight alignment induced in individual molecules by high fields. Today, this alignment has become a source of new structural constraints that, not only leads to better refined structures, but also enables structural characterization of larger molecules and multi-domain molecules.
- For many years, the NMR community argued against high fields for solid state NMR, in part, due to increased requirements for magic angle spinning speeds, and, in part, due to inefficiencies in cross polarization at high field. Sample spinning rates achievable today are an order of magnitude faster than those 15 to 20 years ago, eliminating the former concern. New strategies for cross polarization have met the latter challenge resulting in better spectroscopy over a broad range of field strengths.
- Concerns about decreased resolution in high field microimaging due to bulk magnetic susceptibility effects were frequently expressed. Today, functional magnetic resonance imaging is the direct result of this problem leading to one of the most exciting new techniques in medical imaging.

This is not to say that there are no disadvantages. Indeed, there will be experiments that are better done at low fields, but there are many advantages of very high fields that will lead to multiplicative effects for sensitivity and resolution in many spectroscopy and imaging experiments. Such significant field effects will open new arenas for scientific pursuit.

To illustrate this multiplicative effect, Prof. Grandinetti of Ohio State University compared the magic angle spinning spectra of (6-^{17}O) Methyl α-D-Glucopyranoside at 9.4 T (using a 4 mm rotor spinning at 12 kHz) and at 19.6 T (using a 2.75 mm rotor at 20 kHz). The spectrum obtained at 19.6 T in an hour shows far better resolution and sensitivity than the spectrum obtained at 9.4 T with a week of signal averaging time. The comparison shows a sensitivity enhancement factor of about 20 (or B_o^4) by using high magnetic fields. The B_o^4 factor is a combination of gains through the Boltzman factor, resonance frequency, reduced second-order broadening and resolved spinning side bands by the high magnetic field. Such high field spectra are leading to the development of ^{17}O NMR spectroscopy that has great potential for elucidating chemical and biochemical processes in materials and biological macromolecules.

Advantages of Wide Bore Magnets

Wide bores provide opportunities for increased sample size, gradient coils, and probe components. Adult mice, commonly used for biological studies including gene expression and neurological function, cannot be accommodated in narrow bore magnets with compensated gradient coils and large rf coils are required. Perfused rabbit hearts, commonly used to study relationships between metabolism, structure, function, and mechanics, require a bore greater than 52 mm for imaging. Strong rf fields with excellent homogeneity are needed to excite signals in a strong B_o gradient used for high resolution imaging. They are also needed in solid state NMR, especially for optimal linewidths leading to enhanced sensitivity in magic angle spinning, solid state NMR, and even in solution NMR at high fields where isotropic chemical shift ranges are very large. The ability to achieve such strong rf fields is coupled to the size of the samples and hence the size of the rf coil. Not always is it possible to miniaturize the sample. While *in vivo* specimens are obvious examples, studies of catalytic surfaces, membrane proteins requiring an extensive hydrated lipid bilayer environment, natural abundant samples, dilute environmental samples, or protein preparations that cannot be concentrated may require large sample volumes, and hence high power rf circuitry. Such circuitry requires large size capacitors and insulation to avoid arcing between circuit components. Very low or very high temperature experiments also

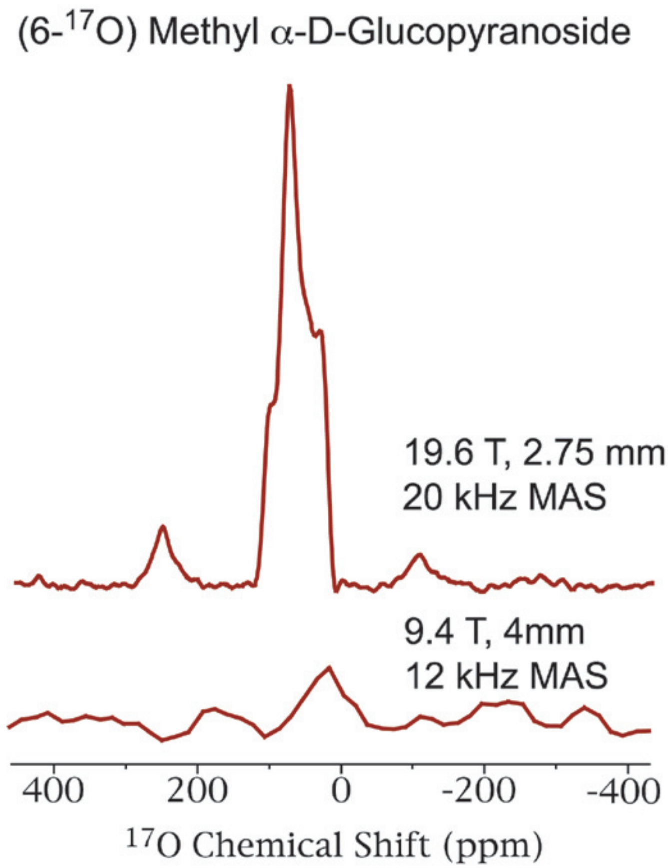

Figure 1. A comparison of magic-angle spinning spectra of ^{17}O labeled sugar using a 400 MHz (9.4 T) instrument at Ohio State Univ. and the 830 MHz (19.6 T) narrow bore spectrometer at the NHMFL. The data collecting time was 1 hour (19.6 T) and 1 week (9.4 T). (P. Grandinetti/Ohio State and Z. Gan/NHMFL).

require considerable space. For magic angle spinning experiments at temperature extremes, not only is space required for the temperature hardware, but also for increased length of the magic angle spinners so that drive and temperature control air can be separated. As a result, there are many NMR spectroscopy and imaging experiments that can take advantage of the 105 mm bore of the NHMFL's unique 900 MHz magnet.

High Field NMR Spectroscopy and Imaging Science Drivers

Structural and Unstructural Biology

- Structural and dynamic characterizations of high molecular weight macromolecules and macromolecular complexes at high fields
- Characterization of nascent structure in weakly structured macromolecular systems

Membrane Proteins

- High resolution structural characterization of membrane proteins
- Enhanced orientation at high fields of samples for solution and solid-state NMR spectroscopy
- ^{1}H solid state spectroscopy and multiple contact spectroscopy of membrane proteins uniquely requiring high fields

Materials Chemistry

- Reduction of second order broadening in quadrupolar nuclei through high fields, multiple quantum magic angle spinning (MQMAS), satellite transition magic angle spinning (STMAS), and double rotation (DOR)
- Low gamma nuclei at high fields, characterizations of metal sites in materials including both high and low temperature extremes
- High resolution solid state NMR through high speed ^{1}H and ^{19}F MAS at high fields

Cellular Ultrastructure and Kinetics

- Improved temporal resolution at high fields for monitoring metabolic and diffusional rates
- Use of intermolecular zero quantum coherences (iZQC) for enhanced contrast in imaging
- Microimaging for ultrastructural characterization and for monitoring diffusional anisotropy

Based on the above advantages of high fields and wide bores, the following science drivers have been assembled as a guide to the user activities on the 105 mm bore 900 at the NHMFL. We encourage users who are particularly interested in pushing the frontiers of the application science or users interested in the development of technology, methodology, or strategies for high field spectroscopy and imaging. The NHMFL has research faculty who are willing to work with you in any of these areas associated with our mission. For more information, please go to our Web site: *http://nmr.magnet.fsu.edu/*.